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**14. ABSTRACT**

Over-expression of LIGHT has been show in various tumor models to induce tumor regression and tumor immunogencity. However, the models are based on transplanted tumors that express artificial foreign antigens that function as tumor antigens, LIGHT has never been evaluated in prostate cancer, where self-antigens likely exist. We have provided the first evidence that LIGHT-induced T cells are specific for at least one relevant prostate expressed self-antigen, PSCA. We have also demonstrated that LIGHT treatment in prostate cancer has a positive effect on the tumor microenvironment, which suggests a strong likelihood that combination treatment with LIGHT and immunotherapeutic vaccination will have an impact against primary and possibly metastatic prostate cancer. Thus, therapeutic intervention by delivering LIGHT to the tumors may serve the dual purpose of inhibiting immune-suppression mediated by regulatory T cells while simultaneously activating tumor-specific immune responses, which we hope to demonstrate can be boosted by vaccination. This study may potentially provide a practical means of overcoming tumor-mediated immunosuppressive mechanisms in a variety of solid human tumors, including those of the prostate, which would have important implications for patients who are diagnosed at the later stages of disease and currently have no recourse for treatment.

**15. SUBJECT TERMS**

Regulatory T cells, prostate cancer, immunosuppression, tumor microenvironment

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# INTRODUCTION

Prostate cancer is the second leading cause of cancer-related death in the United States and according to the American Cancer Society's most recent estimates, will affect almost 200,000 men in 2009. Of these, almost 30,000 men are estimated to die in the United States [1, 2]. Much of the focus of past and current research aims to improve methods to detect the disease at the very earliest stage of carcinogenesis. However, treatment options remain limited [3]. In many cases, expectant management or "watchful waiting" is the standard of care. The current modalities available for prostate cancer treatment have debilitating side effects which include, but are not limited to, urinary, bowel and erectile dysfunction, loss of fertility, effects due to the loss of testosterone (including fatigue, decreased sexual desire, weight gain, loss of muscle mass and osteoporosis) and the well-known devastating side effects of chemotherapy [4, 5]. Metastatic prostate cancer is a death sentence as it is infeasible to remove metastasis by radiation, surgery or any other existing modality. There is no cure for advanced prostate cancer, and thus, there is a significant need to focus research efforts on developing new therapeutic strategies.

While surgery or radiation therapy may be used to treat primary tumors, once the disease spreads beyond the prostate, immunotherapy may be the only way to treat it [6, 7]. A majority of clinical trials for the immunotherapy of prostate cancer have yielded results similar to those seen for most other cancers, which is the induction of tumor-specific immune responses yet limited success in terms of regression or survival. Despite the 2009 U.S Food and Drug Administration (FDA) approval of PROVENGE, the first immunotherapeutic cell-based vaccine that can be prescribed for hormone-refractory prostate cancer patients, excitement is dampened because there have been no objective cures [8]. The failure to clear tumors despite successful induction of immunity in the majority of clinical trials may, in part, be attributed to the suppressive environment within the tumor that disables function of the immune system. Thus, it is essential to develop therapeutic modalities that aim to generate tumor-specific immunity and simultaneously inhibit local immune suppression [9]. Since regulatory T cells appear to be central to inhibiting anti-tumor immunity, **the goal of our proposal is to establish a therapeutic intervention that can overcome the suppressive activity of regulatory T cells while simultaneously inducing prostate cancer-specific immunity.**

LIGHT, a ligand for Herpes Virus Entry Mediator (HVEM) and Lymphotoxin beta-receptor (LTβR), is predominantly expressed on activated immune cells, signaling via LTβR is required for the formation of organized lymphoid tissues while signaling via HVEM induces costimulation [10-13]. Although LIGHT has not been extensively studied in the prostate cancer setting and has not been associated with the inhibition of Treg development or function, our previous experience using LIGHT in a virally-induced tumor model suggests a strong connection between forced LIGHT expression in tumors with a survival benefit and change in tumor milieu [14-16]. Therefore, **we hypothesize that Treg formation and function within the tumor microenvironment can be inhibited by the forced expression of the costimulatory molecule, LIGHT, thereby improving the efficacy of therapeutic vaccines in the absence of a suppressive tumor microenvironment where strong anti-tumoral response may emerge, resulting in an increase survival and tumor specific immunogenicity.** Thus we have proposed the following aims: Aim 1) To determine whether forced expression of LIGHT can inhibit prostate tumor-induced differentiation and function of CD4+ regulatory T cells; Aim 2) To determine whether forced expression of LIGHT can alter the pattern of infiltration and maturation of immune cells, other than T cells, within the tumor microenvironment; Aim 3) To determine whether forced expression of LIGHT in combination with vaccination can induce regression of well-established primary and metastatic prostate tumors.
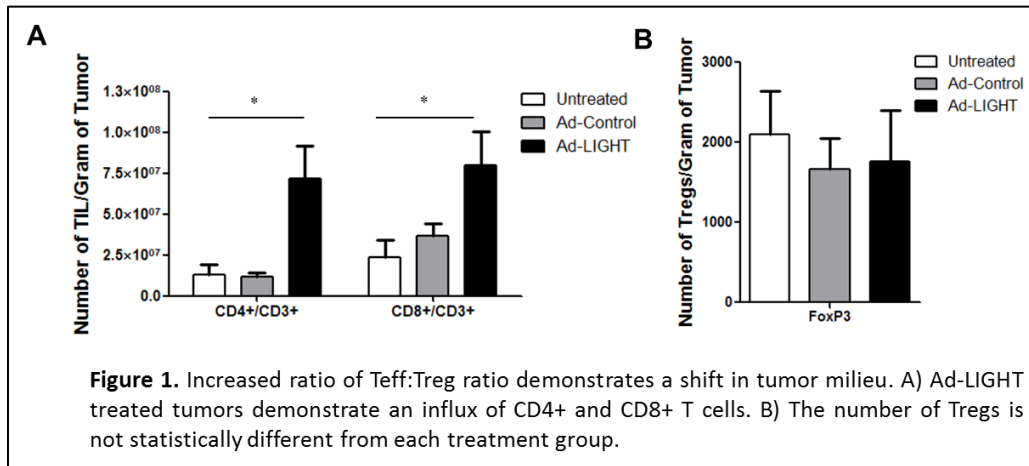
**BODY**

**SPECIFIC AIM 1: Determine whether forced expression of LIGHT can inhibit prostate tumor-induced differentiation and function of CD4+ regulatory T cells.**

**Task 1.1** Compare the effect of treatment with Ad-LIGHT on frequency and function of CD4+ T cells.

Task 1.1 was completed in the last progress report.

**Task 1.2** Determine whether tumors induce differentiation of naïve CD4+ T cells into Tregs



**Figure 1.** Increased ratio of Teff:Treg ratio demonstrates a shift in tumor milieu. A) Ad-LIGHT treated tumors demonstrate an influx of CD4+ and CD8+ T cells. B) The number of Tregs is not statistically different from each treatment group.

There was an increase in intratumoral CD4+ and CD8+ T cells following forced expression of membrane bound LIGHT in a prostate cancer tumor model. (A) Tumor infiltrating lymphocytes were released from untreated or treated tumors 7 days after Ad-Control or Ad-LIGHT injection. Cells were stained with CD4, CD8 and CD3 Ab and analyzed via flow cytometry. The number of TIL/gram of tumor from CD8+/CD3+ and CD4+/CD3+ T cells were significantly higher in Ad-LIGHT treated mice compared to untreated. ($p<0.05$, one-way ANOVA). (B) The number of CD4+CD25+Foxp3+ Tregs per gram of tumor were not significantly differently, despite the increase in total number of infiltrating lymphocytes in the Ad-LIGHT samples. Shown is the average number of FoxP3+ TIL (±SD) from 5 treated mice/group. Data are representative of two individual experiments.

**Task 1.3** Determine whether forced expression of LIGHT in tumor can prevent the differentiation of naïve CD4+ T cells into Tregs.

Task 1.3 was completed in the last progress report.

**Task 1.4** Determine the effect of forced expression of LIGHT on the differentiation and activation state of tumor-infiltrating CD4$^+$ T cells.

We are currently working on Task 1.4.
    *** We have requested a no cost extension to finish up experiments: Task 1.4.


**SPECIFIC AIM 2: Determine whether forced expression of LIGHT can alter the pattern of infiltration and maturation of immune cells, other than T cells, within the tumor microenvironment.**

**Task 2.1** Compare the intra-tumoral cytokines and chemokine profile following treatment with Ad-LIGHT
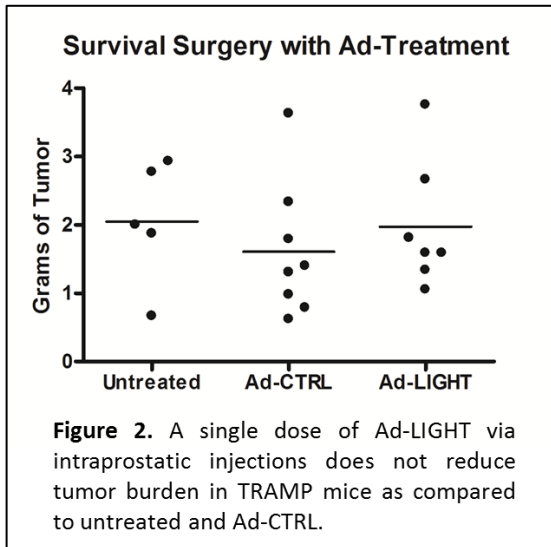
Task 2.1 was complete in the last progress report.

**Task 2.2** Compare the frequency and phenotype of tumor-infiltrating cells.

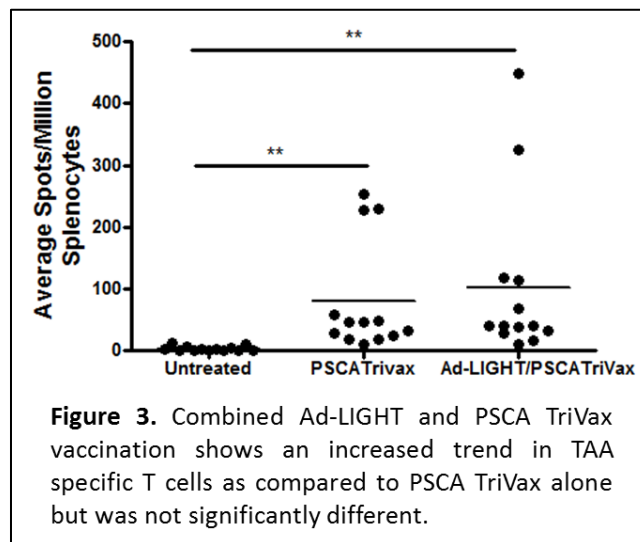Task 2.2 was completed in the last progress report.

**SPECIFIC AIM 3: Determine whether forced expression of LIGHT in combination with vaccination can induce regression of well-established primary and metastatic prostate tumors.**

**Task 3.1** Determine efficacy of treatment with Ad-LIGHT on inducing prostate cancer associated antigen-specific CD8+ T cells and regression of autochthonous primary prostate tumors in TRAMP mice.

**Figure 2.** A single dose of Ad-LIGHT via intraprostatic injections does not reduce tumor burden in TRAMP mice as compared to untreated and Ad-CTRL.

10-12 week old TRAMP mice treated with Ad-LIGHT via intra prostatic injections are not significantly different than Ad-Control or Untreated mice when comparing its tumor burden, harvested at 20 weeks of age. These results indicate that the single treatment of Ad-LIGHT does not induce a strong anti-tumoral response to reduce tumor burden in autochthonous primary tumors. This may suggest that a single dose of LIGHT may not be effective in inducing an immune response to eradicate the existing tumor. Potentially, the effects of LIGHT may have been diminished by the time tumor weights were measured since the length of time between treatment and harvest are approximately 8 weeks apart.

**Task 3.2** Determine efficacy of treatment with Ad-LIGHT on inducing prostate cancer associated antigen-specific CD8+ T cells and regression of primary tumors in mice challenged with TRAMP-C2 cells.

**Figure 3.** Combined Ad-LIGHT and PSCA TriVax vaccination shows an increased trend in TAA specific T cells as compared to PSCA TriVax alone but was not significantly different.

Ad-LIGHT treated mice do not induce TAA specific T cells and does not contribute to the efficacy of PSCA TriVax. This result indicates the the mechanism of action for Ad-LIGHT is merely the recruitment of T cells into the tumor microenvironment and the reduction in functionality of Tregs.

**Task 3.3** Compare efficacy of treatment with Ad-LIGHT and combined treatment of Ad-LIGHT followed by PSCA vaccination in inducing regression of primary tumors in mice with TRAMP-C2 tumors.

Task 3.3 was completed in the last progress report.

**Task 3.4** Determine whether combined treatment of Ad-LIGHT followed by PSCA vaccination induces regression of metastatic tumors in mice challenged with TRAMP-C2 cells.

| Group | TRAMP-C2 Challenge 5x10$^5$ cells | Tumor Resection | TRAMP-C2 re-Challenge 1x10$^6$ cells | % Tumor Regrowth | |
|---|---|---|---|---|---|
| Naïve | --- | --- | 5/5 | 100% | |
| Untreated | 5/5 | 4/4 | 3/3 | 100% | *1 mouse censored |
| Ad-LIGHT | 5/5 | 5/5 | 3/4 | 75% | *1 mouse censored |
| Ad-Control | 5/5 | 3/3 | 3/3 | 100% | |
| PSCA TriVax | 5/5 | 5/5 | 1/5 | 20% | |
| Ad-LIGHT/PSCATriVax | 5/5 | 4/4 | 1/3 | 33% | *1 mouse censored |
| Ad-Control/PSCA TriVax | 5/5 | 4/4 | 1/4 | 25% | |

**Table 1.** TrampC2 challenged mice were treated as seen in the groups above. Tumors were resected 1 weeks post treatment and the mice were rechallenged with 1x10$^6$ cells 2 weeks post resection. The % tumor regrowth evaluates whether the rechallenged flank had tumor growth.

In this study, C56BL6 mice were first challenged with 5x10$^5$ TrampC2 cells, normalized when tumor volumes were approximately 30 and treated with the appropriate vaccination scheme according to each group. Tumors were then resected 1 week post treatment and rechallenged 2 weeks post resection. Mice with resected tumors that grew back were censored in the study. The induction of memory T cells plays an essential role in cancer-fighting properties. The idea of a successful vaccine provides an advantage to the immune system in mounting an immune response against invasive diseases and pathogens. Memory T cells plays a crucial role in orchestrating these immune responses, they have distinct activation and intracellular markers with a lower threshold and a diverse cytokine, profile against specific antigens [17-19]. Some studies show that *in-situ* tumor destruction of melanoma or fibrosarcomas (excision of the primary tumor), aid the immune system in mounting an anti-tumoral response against the tumor re-challenge [20, 21]. In this study, we show that the excision of the primary tumor did not protect against the tumor re-challenge (untreated group). However, we demonstrate that the vaccination with PSCA TriVax and subsequently the removal of the primary tumor, protected against the tumor re-challenge. This data suggest that PSCA TriVax induced tumor antigen specific immune response that were capable of mounting an immune response against TRAMP-C2 cells. In a translational sense, patients diagnosed with prostate cancer may opt for a prostatectomy in combination with a therapeutic vaccine that will further control the future progression of the malignant disease [22]. LIGHT was not capable of inducing memory against TRAMP tumors, although this was not surprising due to the lack of TAA specific T cells in LIGHT vaccinated mice.

**Task 3.5** Determine whether combined treatment of Ad-LIGHT followed by PSCA vaccination prevents the outgrowth of spontaneous metastatic tumors in TRAMP mice.

10 week old TRAMP mice were vaccinated and treated with either Ad-LIGHT, Ad-Control or no treatment via intra-prostatic injections. The mice were then sacrificed and tumor weights were harvested at 20 weeks of age to evaluate tumor burden. The TRAMP model is widely used in the field of prostate cancer research, since the progression of disease mirrors a subset of patient cases. In this study, the vaccination of TRAMP mice with PSCA TriVax, Ad-Control and Ad-LIGHT alone did not improve the disease status. However the combination of PSCA TriVax and an adenovirus vector (either Ad-Control or Ad-LIGHT) induced an immunogenic

response and resulted in a lower tumor burden as compared to single treatments, suggesting an adenovirus effect. Our results propose that the use of an immunogenic vaccine with an inflammatory response via intraprostatic injections may reduce tumor burden. The adenovirus effect parallels our results from the mRNA of IDO, NOS and Arg2 (From Task 2.2 Second Annual Report) where an adeno inflammation effect was detected. (***$p < 0.001$, one-way ANOVA).
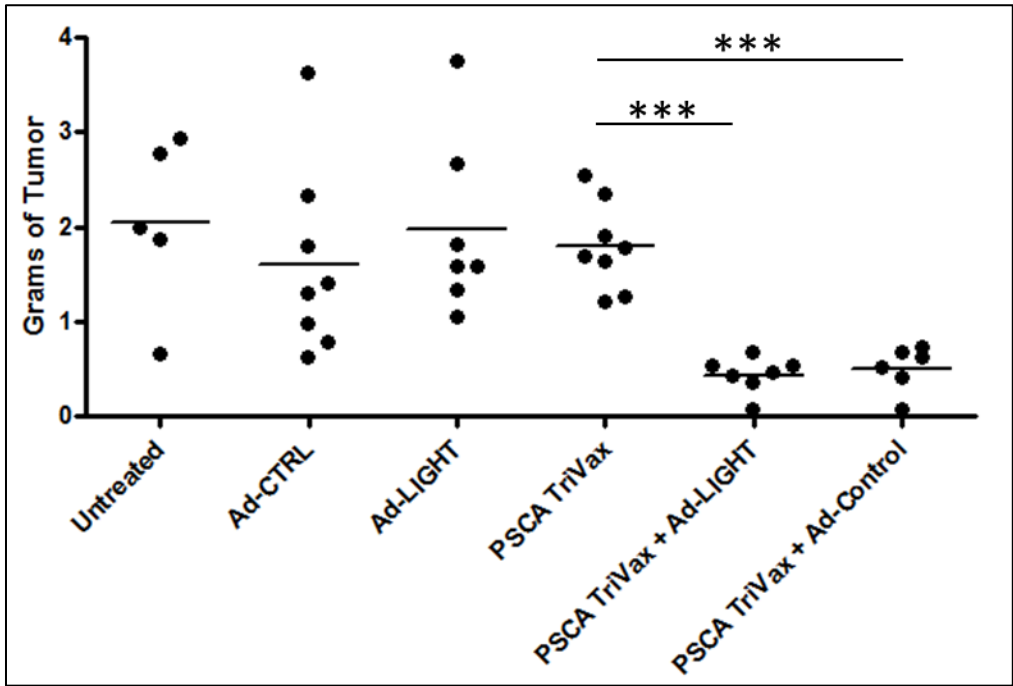


**Figure 4.** Adeno-virus synergistically reduces tumor burden in PSCA vaccinated mice. LIGHT itself does not contribute to this effect.

**KEY RESEARCH ACCOMPLISHMENTS**

- The number of T cells is significantly higher in Ad-LIGHT treated tumors than Ad-Control or Untreated.
- Interestingly, the number of Tregs is not statistically different in each treatment group.
- PSCA TriVax induces TAA specific T cells, LIGHT aids in the recruitment of naïve T cells to the tumor microenvironment.
- PSCA TriVax induces memory T cells in a tumor re-challenge experiment.
- TRAMP mice have a reduced tumor burden when treated with PSCA TriVax and Ad-LIGHT or Ad-Control, indicating an adeno inflammation effect.

**REPORTABLE OUTCOMES**

1. Yan, L. Da Silva, DM., Verma, B., Gray, A., Brand, H., Skeate, J., Porras, T., Kanodia, S., Kast., W.M. **Forced LIGHT expression in prostate tumors overcome Treg mediated immunosuppression and synergizes with a prostate tumor therapeutic vaccine by recruiting effector T lymphocytes.** *The Prostate.* – In Press.
2. Yan, L., Trainee, 2014 ASM Scientific Writing & Publishing Institute (SWPI), March 20-23, 2014, Washington, D.C.

**CONCLUSION**

Our results provide evidence that the treatment of prostate tumors with LIGHT can synergize with a therapeutic cancer vaccine in enhancing the anti-tumor response through the recruitment of immune modulating T cells, while Tregs are not being recruited, resulting in a positive switch in the Teff/Treg balance. LIGHT does not contribute to the induction of TAA specific T cells or the development of memory T cells.

# REFERENCES

1. Gronberg, H., *Prostate cancer epidemiology.* Lancet, 2003. **361**(9360): p. 859-64.
2. Jemal, A., et al., *Cancer statistics, 2002.* CA Cancer J Clin, 2002. **52**(1): p. 23-47.
3. Adamo, V., et al., *Emerging targeted therapies for castration-resistant prostate cancer.* Front Endocrinol (Lausanne), 2012. **3**: p. 73.
4. Zaider, T., et al., *Loss of Masculine Identity, Marital Affection, and Sexual Bother in Men with Localized Prostate Cancer.* J Sex Med, 2012.
5. Fitzpatrick, J.M., *Management of localized prostate cancer in senior adults: the crucial role of comorbidity.* BJU Int, 2008. **101 Suppl 2**: p. 16-22.
6. Boikos, S.A. and E.S. Antonarakis, *Immunotherapy for prostate cancer enters its golden age.* Clin Med Insights Oncol, 2012. **6**: p. 263-73.
7. Rosenberg, S.A., *Progress in human tumour immunology and immunotherapy.* Nature, 2001. **411**(6835): p. 380-4.
8. Bulloch, M.N., M.M. Elayan, and H.R. Renfroe, *Sipuleucel-T: a therapeutic cancer vaccine for the treatment of castration- or hormone-refractory prostate cancer.* Expert Rev Clin Pharmacol, 2011. **4**(6): p. 685-92.
9. Mills, K.H., *Regulatory T cells: friend or foe in immunity to infection?* Nat Rev Immunol, 2004. **4**(11): p. 841-55.
10. Stanley, A.C., et al., *Critical Roles for LIGHT and Its Receptors in Generating T Cell-Mediated Immunity during <italic>Leishmania donovani</italic> Infection.* PLoS Pathog, 2011. **7**(10): p. e1002279.
11. Zhu, M. and Y.-X. Fu, *The role of core TNF/LIGHT family members in lymph node homeostasis and remodeling.* Immunological Reviews, 2011. **244**(1): p. 75-84.
12. Kojima, R., et al., *Molecular Basis for Herpesvirus Entry Mediator Recognition by the Human Immune Inhibitory Receptor CD160 and Its Relationship to the Cosignaling Molecules BTLA and LIGHT.* Journal of Molecular Biology, 2011. **413**(4): p. 762-772.
13. Doherty, T.A., et al., *The tumor necrosis factor family member LIGHT is a target for asthmatic airway remodeling.* Nat Med, 2011. **17**(5): p. 596-603.
14. Kanodia, S., et al., *Expression of LIGHT/TNFSF14 combined with vaccination against human papillomavirus Type 16 E7 induces significant tumor regression.* Cancer Res, 2010. **70**(10): p. 3955-64.
15. Lasaro, M.O. and H.C. Ertl, *Targeting inhibitory pathways in cancer immunotherapy.* Curr Opin Immunol, 2010. **22**(3): p. 385-90.
16. Apostolou, I., et al., *Peripherally induced Treg: mode, stability, and role in specific tolerance.* J Clin Immunol, 2008. **28**(6): p. 619-24.
17. Lanzavecchia, A. and F. Sallusto, *Antigen decoding by T lymphocytes: from synapses to fate determination.* Nat Immunol, 2001. **2**(6): p. 487-92.
18. Butcher, E.C., et al., *Lymphocyte trafficking and regional immunity.* Adv Immunol, 1999. **72**: p. 209-53.
19. Campbell, J.J. and E.C. Butcher, *Chemokines in tissue-specific and microenvironment-specific lymphocyte homing.* Curr Opin Immunol, 2000. **12**(3): p. 336-41.
20. Normann, S., M. Schardt, and E. Sorkin, *Cancer induced anti-inflammation and its potentiation by tumor excision and rechallenge.* J Leukoc Biol, 1987. **42**(1): p. 61-8.
21. den Brok, M.H., et al., *In situ tumor ablation creates an antigen source for the generation of antitumor immunity.* Cancer Res, 2004. **64**(11): p. 4024-9.
22. Rosenberg, S.A., *Cancer vaccines based on the identification of genes encoding cancer regression antigens.* Immunol Today, 1997. **18**(4): p. 175-82.